ISSN 0825-4591

PROCEEDINGS

ENVIRONMENTAL RESEARCH:
1989 TECHNOLOGY TRANSFER CONFERENCE

November 20 & 21, 1989

Royal York Hotel

Toronto, Ontario

VOLUME I

FEATURE PRESENTATIONS

AIR QUALITY RESEARCH

WATER QUALITY RESEARCH

Sponsored by

Research and Technology Branch

Environment Ontario

Ontario, Canada

8 4

BP5

MICROBIAL METHYLATION OF MERCURY IN ACID STRESSED LAKES - ROLE OF SULPHATE REDUCING BACTERIA

Alison Kerry[*1], Pamela M. Stokes[2], Betsy Prucha[3], and Greg Mierle[3]
[1]BIOS Environmental Research, 344 Quebec Ave., Toronto, Ontario, M6P 2V3
[2]Institute for Environmental Studies, University of Toronto, Toronto, Ont., M5S 1A4
[3]Dorset Research Centre, O.M.O.E., Bellwoods Acres Rd., Dorset, Ont., P0A 1E0.

INTRODUCTION

Elevated levels of mercury have recently been detected in fish from acid stressed and other softwater lakes of the Muskoka-Haliburton region in south-central Ontario, Canada. 95% of the lakes in this region contain sport fish that are above the guideline for unrestricted human consumption. There is no clear source of mercury contamination in this area and thus no abatement measures have been implemented. This non-point source mercury contamination may be due, in part, to long range atmospheric transport of mercury from combustion sources.

Some of the occurrences of the elevated mercury levels in fish tissue, in lakes removed from point sources, appear to be related to the acidity of surface waters (Sloan & Schofield, 1983; Wiener, 1983; Wren & MacCrimmon, 1983; Bjorkland et al., 1984; Suns et al., 1987; Hakanson et al., 1988). Other factors, such as the supply of organic carbon (Bodaly et al., 1984; Hecky et al., 1987; Jackson, 1988), the form and availability of mercury (Rudd & Turner, 1983), the presence of humic and fulvic acids (Mannio et al., 1986; Surma-Aho et al., 1986), and the amount of sulphate present (Compeau & Bartha, 1985; Gilmour & Mitchell, in press), are also statistically related to the level of fish mercury.

Mercury readily accumulates in muscle as well as other tissue of fish and is biomagnified in the aquatic food chain (Huckabee et al., 1979). Methylation of mercury by microorganisms produces a form of mercury that is more toxic and more biologically available to biota than is inorganic mercury (Beijer & Jernelov, 1979). Microbial communities in lake sediment are known to methylate inorganic mercury and logically, factors which change or stimulate portions of this community can influence that rate of methylation (Summers & Silver, 1978). It has been suggested, based on estuarine studies, that one group of sediment microbes, the sulphate-reducing bacteria, may be significant methylators of mercury (Compeau and Bartha, 1985). It was previously proposed that methanogenic bacteria were responsible for the major amount of mercury methylation (Wood et al., 1968; Jensen & Jernelov, 1968).

The increased input of sulphate from acidic deposition may stimulate sulphate-reducing bacteria over the other sediment populations thereby potentially increasing the rate of methylmercury production in the system. The objective of our study was to elucidate the possible role of freshwater sulphate-reducing bacteria in methylmercury production and to determine the effect that sulphate input can have on their activity. The role of freshwater sulphate-reducing bacteria from the Muskoka-Haliburton area (Dickie Lake, pH 5.7) in methylmercury production was examined by adding specific microbial inhibitors to anoxic lake sediments spiked with $^{203}HgCl_2$. The effect of increased sulphate on the activity of sulphate-reducing bacteria both in terms of sulphate reduction rate and methylation of mercury was examined by adding appropriate ranges of concentrations of sulphate to $^{203}HgCl_2$ spiked sediments.

MATERIALS AND METHODS

Dickie Lake in the Muskoka-Haliburton region of south-central Ontario, Canada (Latitude 45° 09'N, Longitude 79° 05'W) was sampled regularly to provide sediment and water. The pH of the lake ranged from pH 5.7 to 6.0 with an acid shock event (pH 5.1) at snowmelt. The sediments were sandy with low organic content (Loss on Ignition, 450°C for 4 hrs, was 1-4%) and the lake water had a sulphate concentration of 6-8 $mg.L^{-1}$.

Sediments were collected from the epilimnion near the outflow of Dickie lake. Cores (10 by 50 cm) were taken by hand at a depth of 1-2 m in replicates of six. The cores were then packed on ice and transported to the laboratory where they were preincubated at 25°C for 2 weeks before use. Cores were extruded and manipulated in an anaerobic glove box filled with flowing oxygen-free nitrogen gas. After the top 1 cm of floc had been removed, the subsequent 5-10 cms of anoxic sediments were pooled from at least 3 cores. Sediment slurries were made with a medium designed to approximate the low-alkalinity waters of acid sensitive lakes in the study area, Muskoka medium (Wehr and Brown, 1985), with the following modifications: sulphate content was lowered to 3 $mg.L^{-1}$, vitamins were eliminated, and 0.5 $g.L^{-1}$ of yeast extract was added. A slurry was made of 1 part sediment to 3 parts Muskoka medium. Forty ml aliquots were dispensed into 60 ml serum bottles and sealed with butyl rubber stoppers (Compeau and Bartha, 1985).

All reagents were then added by syringe into the sealed bottles outside the anaerobic glove box through the respective septa.

Specific rates of mercury methylation were determined using the radiochemical method of Furutani and Rudd (1980). 1 µCi of $^{203}HgCl_2$, approximately 1 µg of Hg, was added to each 40 ml sample bottle through the septum (approx. 67 ng per dry sediment) and incubated at 25°C. At the end of the experiment, 1 ml of 4N HCl was added to stop the biological reaction and alkylated ^{203}Hg was extracted from the total contents of the bottle (Furutani and Rudd, 1980). The alkylated ^{203}Hg, extracted into toluene, was then dried with Na_2SO_4 to remove excess water and determined on a gamma counter. All experiments were done with at least 3 replicates at each condition along with three acid-killed blanks (1 ml of 4N HCl added to bottle). The extraction efficiency of the technique was assessed by using the methylated isotope, $CH_3^{203}Hg$; extraction was 78-80% efficient. All calculations were corrected accordingly.

RESULTS & DISCUSSION

Both sulphate-reducing bacteria and methanogens were present in the anoxic sediment of Dickie Lake. Specific microbial inhibitors, that were added to ^{203}Hg spiked slurries, were effective in inhibiting the target microbial population, i.e. $NaMoO_4$ inhibited sulphate-reducing bacteria and BESA inhibited methanogenic bacteria. $NaMoO_4$ decreased methyl-mercury production by 75% but BESA did not significantly decrease methylmercury production (Figure 1). Varying levels of inhibitors; 5, 10, 20 mM NaMo and 7.5, 15, 30 mM BESA, produced the same pattern as shown in Figure 1 independent of concentration. This indicates that sulphate-reducing bacteria are the main methylators of mercury in these sediments under the experimental conditions that are used.

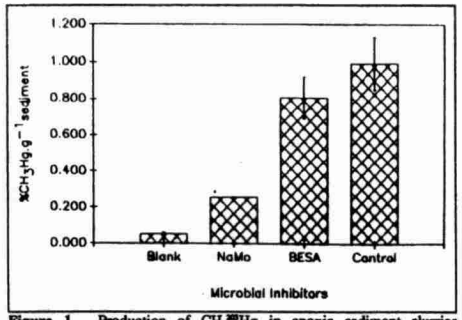

Figure 1. Production of $CH_3^{203}Hg$ in anoxic sediment slurries (Blank = Acid killed, NaMo = 10 mM Na_2MoO_4, BESA = 15 mM 2-Bromoethane sulfonic acid,Control = no additions).

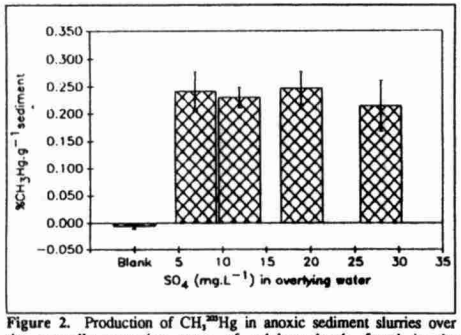

Figure 2. Production of $CH_3^{203}Hg$ in anoxic sediment slurries over the naturally occurring range of sulphate levels found in the experimental area.

The rate of sulphate reduction from the water overlying the sediment slurries increased as the concentration of sulphate in the water increased. This indicates that the resident population of sulphate-reducing bacteria are stimulated by small inputs in sulphate to the water column. However, the increase in activity of the sulphate-reducers did not correlate with increased production rates of methylmercury. Across the range of sulphate concentrations tested, there was no significant difference in the amount of methylmercury produced (Figure 2). Therefore the stimulatory effect of sulphate on these bacteria does not seem to be translated into increased methylation rates of mercury.

CONCLUSIONS

Under our experimental conditions, sulphate-reducing bacteria do appear to be major methylators of mercury in freshwater, anoxic sediments and were more important in this respect than methanogens. However, the stimulatory effect of sulphate, within normal levels, on these bacteria's ability to further methylate mercury remains unclear.

ACKNOWLEDGMENTS

We would like to thank the Ontario Ministry of the Environment for funding this project. Thanks also go to Brenda Miskimmin, Miriam Diamond, SLOWPOKE reactor facility, Dr. T.C. Hutchinson, Matt Hilgerdenaar, and Dr. G. Mierle for all his intellectual and technical input.

LITERATURE CITED

Beijer, K., and A. Jernelov. 1979. p. 203-210. In J.O. Nriagu (ed.), The biogeochemistry of mercury in the environment. Elsevier/North-Holland Biomedical Press, Amsterdam.

Bjorkland, I., H. Borg, and K. Johansson. 1984. Ambio. 13:118-121.

Bodaly, R.A., R.E. Hecky, and R.J.P. Fudge. 1984. Can. J. Fish. Aquat. Sci. 41:682-691.

Compeau, G.C., and R. Bartha. 1985. Appl. Environ. Microbiol. 50:498-502.

Furutani, A., and J.W.M. Rudd. 1980. Appl. Environ. Microbiol. 40:770-776.

Hakanson, L., A. Nilsson, and T. Andersson. 1988. Environ. Pollut. 49:145-162.

Hecky, R.E., R.A. Bodaly, D.J. Ramsay, P.S. Ramlal, and N.E. Strange. 1987. In Report for the Canada-Manitoba agreement of the study and monitoring of mercury in the Churchill River Diversion.

Huckabee, J.S., J.W. Elwood, and S.G. Hildebrand. 1979. p.277-231. In J.O. Nriagu (ed.), The biogeochemistry of mercury in the environment. Elsevier/North-Holland Biomedical Press, Amsterdam.

Jackson, T.A. 1988. Can. J. Fish. Aquat. Sci. 45:97-121.

Jensen, S., and A. Jernelov. 1969. Nature (London) 223:753-754.

Mannio, J., M. Verta, P. Kortelainen, and S. Rekolainen. 1986. Publications of Water Research Institute, National Board of Waters, Finland. No. 65:32-43.

Rudd, J.W.M., and M.A. Turner. 1983. Can. J. Fish. Aquat. Sci. 40:2206-2217.

Sloan, R., and C. Schofield. 1983. N. East. Environ. Sci. 2:165-170.

Summers, A.O., and S. Silver. 1978. Ann. Rev. Microbiol. 32:637-672.

Suns, K., G. Hitchin, B. Loescher, E. Pastorek, and R. Pearce. 1987. Water Resources Branch, Ontario Ministry of the Environment.

Surma-Aho, K., J. Paasivirta, S. Rekolainen, and M. Verta. 1986. Chemosphere. 15:353-372.

Wehr, J.D., and L. M. Brown. 1985. Can. J. Fish. Aquat. Sci. 42:1783-1788.

Wiener, J.G. 1983. FWS/OBS-80/40.16, US Fish and Wildlife Service. Eastern Energy and Land Use Team.

Wood, J.M., F.S. Kennedy, and C.G. Rosen. 1968. Nature (London) 220:173-174.

Wren, C. and H. MacCrimmon. 1983. Can. J. Fish. Aquat. Sci. 40:1737-1744.